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NEW 1,4-DIHYDRO[1,8]NAPHTHYRIDINE DERIVATIVES AS DNA GYRASE INHIBITORS

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ABSTRACT

Owing to the growing need for novel antibacterial agents, we synthesized a novel series of fluoroquinolones including 7-substituted-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro[1,8]naphthyridine-3-carboxylic acid derivatives, which were tested against clinically relevant Gram positive and Gram negative bacteria. Chemical structures of the synthesized compounds were identified using spectroscopic methods. *In vitro* antimicrobial effects of the compounds were determined via microdilution assay. Microbiological examination revealed that compounds **13** and **14** possess a good antibacterial profile. Compound **14** was the most active and showed an antibacterial profile comparable to that of the reference drugs trovafloxacin, moxifloxacin, and ciprofloxacin. A significant MIC_{90} value (1.95 µg/mL) against *S. aureus* ATCC 25923, *E. coli* ATCC 35218, and *E. coli* ATCC 25922 was recorded for compound **14**. We observed reduced metabolic activity associated with compounds **13** and **14** in the relevant bacteria via a luminescence ATP assay. Results of this assay supported the antibacterial potency of compounds **13** and **14**. An *E. coli* DNA gyrase inhibitory assay indicated that compound **14** is a potent inhibitor of *E. coli* DNA gyrase. Docking studies revealed that there is a strong interaction between compound **14** and the *E. coli* DNA gyrase enzyme. Genotoxicity and cytotoxicity evaluations of compounds **13** and **14** showed that compound **14** is non-genotoxic and less cytotoxic compared to the reference drugs (trovafloxacin, moxifloxacin, and ciprofloxacin), which increases its biological importance.

Keywords: Fluoroquinolones, DNA gyrase, Antibacterial activity, ATP luminescence, NIH/3T3, Ames MPF

Quinolones are potent antibacterial agents used in the treatment of a wide range of bacterial infections. The history of quinolones started with the discovery of nalidixic acid in 1962. Later, first generation quinolones such as enoxacin, lomefloxacin, and norfloxacin, were discovered.[1] The second, third, and fourth generation quinolones are called fluoroquinolones since they have a fluorine atom attached to the central ring system.[2] Among fluoroquinolones, the most used agents, such as ciprofloxacin, levofloxacin, and moxifloxacin, were developed with a broad-spectrum of activity against Gram negative and Gram positive bacteria (including enterococci, streptococci, and staphylococci).[3] They also have significant activity against most *Enterobacteriaceae* bacteria and against many bacterial species which are multi-resistant to beta-lactam and aminoglycoside antibiotics.[4-6] Fluoroquinolones are capable of inhibiting bacterial growth by interacting with essential DNA replication enzymes, type II topoisomerases, DNA gyrase, and topoisomerase IV.[7]

In order to facilitate the replication of bacterial DNA, DNA gyrase inserts the negative supercoil into DNA that is bound to initiation proteins. Likewise, topoisomerase IV plays a role in DNA segregation by removing the links in the replication forks.[8,9] Although fluoroquinolones are extremely successful antibacterial agents, due to their extensive usage, fluoroquinolone-resistant bacteria have inevitably developed. Antibiotic resistance is a dramatically growing public concern, and the risk of patients developing untreatable infections is quite high. To overcome this challenge, extensive research is being conducted on the synthesis of novel antibiotics. Research is also being conducted on introducing structural modifications in existing antibiotics. Based on these strategies, several drugs have been discovered and improved. As the most practical solution, the second strategy attracts more attention than the first does.[10,11]

The chemical structure of fluoroquinolones comprises a 4-quinolone/1,8-naphthyridone-3-carboxylic acid core and a secondary amino moiety at the C-7 position.[12] A

fluorine atom is attached to C-6 and the substituent at C-7 renders fluoroquinolones their important role in antibacterial effectiveness, spectrum, and safety.[13] The C-7 position of the central ring is known as the most suitable site for chemical change, and the presence of five- or six-membered cyclic amines, such as pyrrolidine, piperazine, and piperidine, at this position are generally characteristic of fluoroquinolones. Ciprofloxacin, levofloxacin, moxifloxacin, gemifloxacin, and trovafloxacin (**Figure 1)** have the most common cores and structures of important fluoroquinolones.[14]

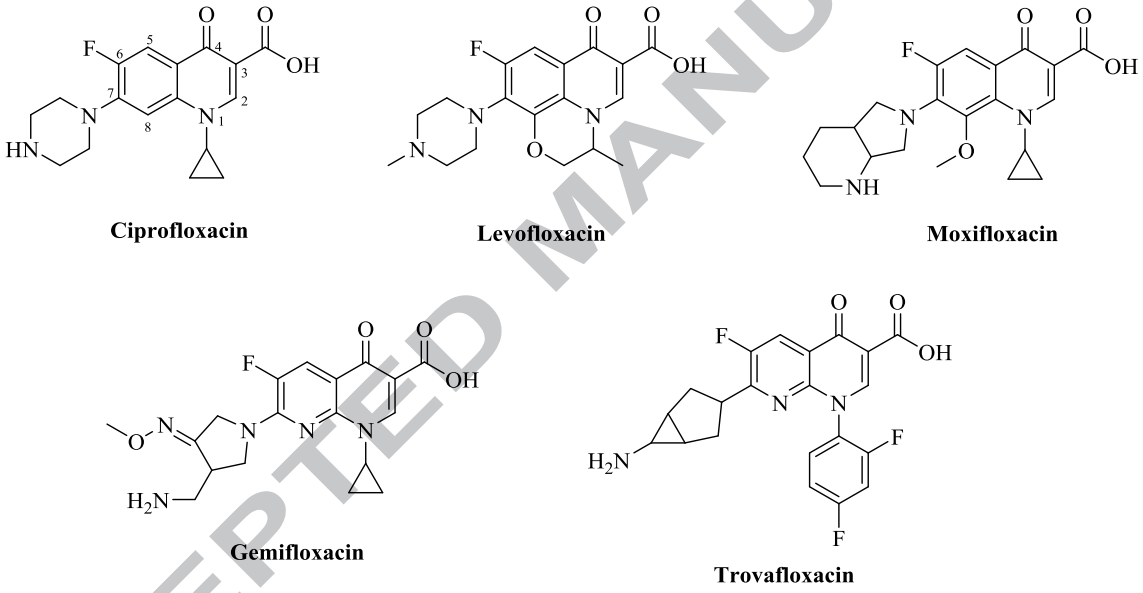
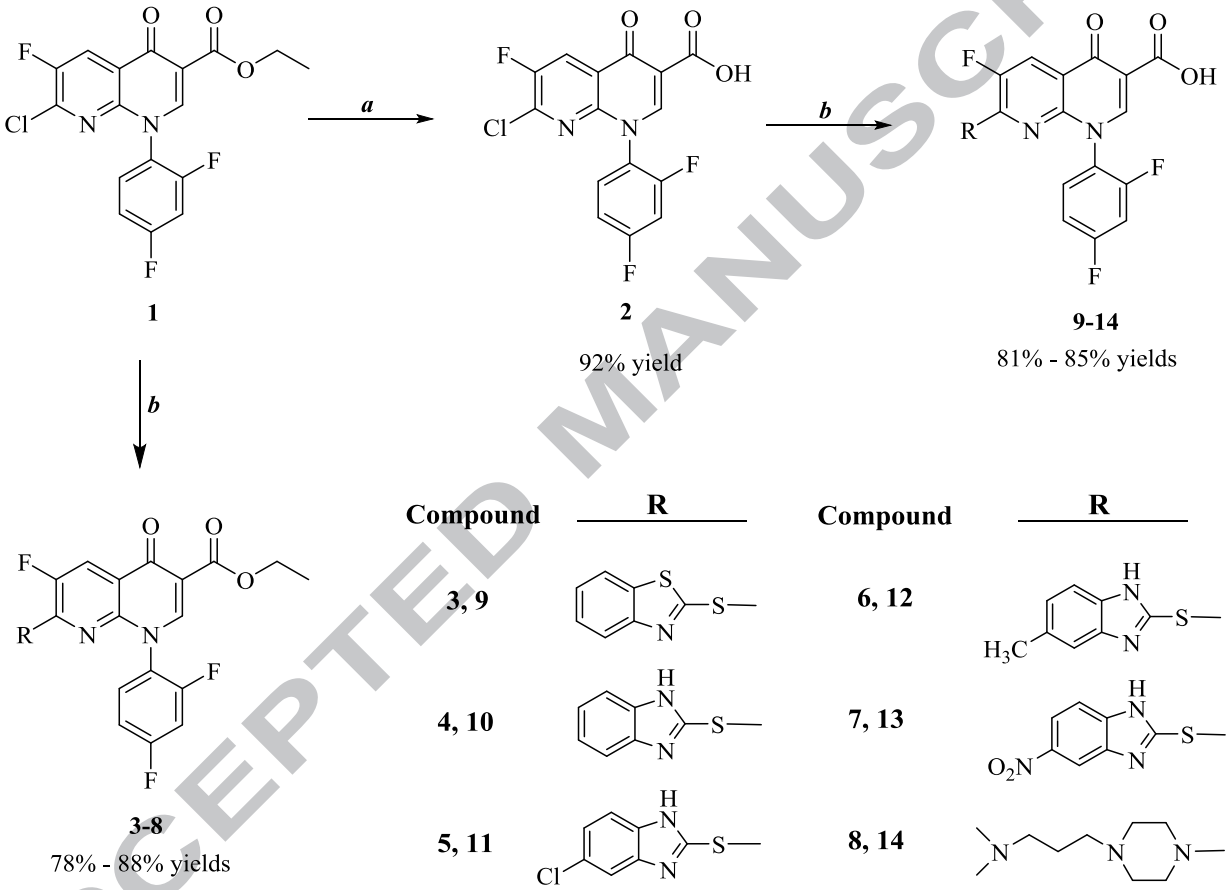


Figure 1: Chemical structures of some main fluoroquinolones and the synthesized compounds (**3-14**).

Despite their success against infectious diseases, fluoroquinolone-type antibiotics have some problematic issues. Multidrug resistance, in some cases, has been attributed to mutations leading to decreased drug permeability of fluoroquinolones.[15] In addition, the effects of quinolones such as plasmid curability, promotion of reversions and forward mutations, and positive results in genotoxicity assays[16-20] suggest the possible bacterial mutagenicity of quinolones. Furthermore, induction of DNA damage in both prokaryotic and eukaryotic cells, and induction of mutations into DNA has been reported as an essential risk

for fluoroquinolones.[21] New fluoroquinolones that do not possess mutagenic potency may help eliminate these problematic issues.

Based on the above information, we synthesized a novel series of compounds in this class to obtain effective and non-mutagenic derivatives (**Scheme 1**.[22]).



Scheme 1. Synthesis pathway of the compounds **3-14**. Reagents and conditions: ***a***: i) 10% HCl, 100 °C, 3 h, ii) adjusted to pH=7 with NH_4OH (33%); ***b:*** i) Appropriate benzazole-2-thiol or 4-(3-dimethylaminopropyl)piperazine, K_2CO_3, Acetone, 40 °C, 12 h, ii) adjusted to pH=7 with AcOH (100%).

Synthesized compounds (**3-14**) were evaluated for antimicrobial activity against various Gram positive (*S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, and *L. monocytogenes* ATCC 7644) and Gram negative bacteria (*E. coli* ATCC 35218, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC

27853). MIC_{90} values were evaluated via fluorometric measurements, using resazurin solution.[23] Moxifloxacin, trovafloxacin, and ciprofloxacin were used as standard drugs in the activity test. Microdilution test results are presented in **Table 1**.

Compounds **3-7** did not show strong activity against bacterial strains, while stronger antibacterial activity was observed for compounds **9-14**. *S. aureus* ATCC 25923, *E. coli* ATCC 35218, and *E. coli* ATCC 25922 bacterial strains were more sensitive to these compounds. Compounds **13** and **14** were the most potent members of the series. Compound **14** was the most active and showed antibacterial activity comparable to that of the reference drugs, moxifloxacin, trovafloxacin, and ciprofloxacin. A significant MIC_{90} value (1.95 µg/mL) against *S. aureus* ATCC 25923, *E. coli* ATCC 35218, and *E. coli* ATCC 25922 was recorded for compound **14**. This compound also showed strong antibacterial activity against *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 with a MIC_{90} value of 3.9 µg/mL, and against *K. pneumoniae* ATCC 700603, *L. monocytogenes* ATCC 7644 and *P. aeruginosa* ATCC 27853 with a MIC_{90} value of 7.8 µg/mL.

There was a significant difference in both the structures and the antibacterial activity profiles of compounds **3**-**8** and **9**-**14**. The initial six compounds bear an ethyl carboxylate moiety at C-3 of the central ring, whereas the following six compounds carry a carboxylic acid group at the same position. Hence, it is clear that esterification of the 1,8-naphthyrid-4-one-3-carboxylic acid core causes a dramatic loss in the antibacterial activity. Compound **9** includes benzothiazol-2-thiol as a substituent at seventh position. On the contrary, compounds **10**-**13** bear a 5-substituted-benzimidazole-2-thiol substitution at the same position. It can be stated that benzimidazole-2-thiol has more impact on antibacterial activity compared to the effect of compound **9**. Furthermore, the 5-nitro-benzimidazol-2-thiol substitution at C-7 of compound **13** enhances antibacterial activity. This may be explained by the DNA-gyrase inhibitory potential of the nitro substituent.[24] The most active compound **14** bears a 4-

dimethylaminopropylpiperazin-1-yl substituent instead of benzazole-2-thiol in the compounds **9-13**. This structural difference resulted in a significant increase in antibacterial activity. Thus, it can be revealed that the presence of a nitrogenous cyclic amine at the C-7 position of 1,8-naphthyrid-4-one-3-carboxylic acid core causes greater enhancement in antibacterial activity than does the presence of thiol substituents at the same position. Thus, the structure activity relationship determined in this study is in an agreement with those of previous studies, [11-14] affirming that carboxylic acid group at C-3 and cyclic secondary amine at C-7 position of 1,8-naphthyrid-4-one main structure are the significant chemical requirements for intrinsic antibacterial activity.

Table 1. Antimicrobial activity (MIC_{90} µg/mL) of compounds 3-14 and reference drugs against pathogenic microorganisms

	Microorganism (MIC_{90} µg/mL)							
Compounds	***S.a***	***E.c 1***	***E.c 2***	***K.p***	***E.f 1***	***E.f 2***	***L.m***	***P.a***
3	62.5	125	125	125	125	125	125	125
4	125	125	62.5	125	125	62.5	125	125
5	62.5	125	62.5	125	125	125	62.5	125
6	62.5	250	125	125	125	250	125	62.5
7	125	125	250	125	250	250	125	125
8	62.5	125	125	250	125	62.5	125	125
9	15.6	31.25	31.25	31.25	31.25	62.5	31.25	31.25
10	7.8	15.6	15.6	31.25	15.6	31.25	31.25	31.25
11	7.8	15.6	15.6	31.25	31.25	31.25	15.6	31.25
12	3.9	3.9	7.8	31.25	31.25	31.25	31.25	15.6
13	1.95	3.9	3.9	15.6	7.8	15.6	15.6	15.6
14	1.95	1.95	1.95	7.8	3.9	3.9	7.8	7.8
Control 1	≤ 1.95	≤ 1.95	≤ 1.95	3.9	≤ 1.95	≤ 1.95	3.9	3.9
Control 2	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95	3.9	≤ 1.95
Control 3	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95	≤ 1.95

The microbial organisms used are ***S.a***: *S. aureus* (ATCC 25923), ***E.c 1***: *E. coli* (ATCC 35218), ***E.c 2***: *E. coli* (ATCC 25922), ***K.p***: *K. pneumoniae* (ATCC 700603), ***E.f* 1**: *E. faecalis* (ATCC 29212), ***E.f 2***: *E. faecalis* (ATCC 51299), ***L.m***: *L. monocytogenes* (ATCC 7644) and ***P.a***: *P. aeruginosa* (ATCC 27853), Control 1: Moxifloxacin, Control 2: Ciprofloxacin, Control 3: Trovafloxacin.

Among various screening methods for in vitro antibacterial activity, adenosine triphosphate (ATP) bioluminescence assay, based on the amount of ATP present, is directly proportional to the number of cells. Therefore, this assay allows for quantification of microbial population in the cell.[25] In order to support the results of the broth microdilution test, effects of the compounds **13** and **14** against *S. aureus* ATCC 25923, *E. coli* ATCC 35218, and *E. coli* ATCC 25922 were investigated via ATP bioluminescence assay. A concentration of 1.95 µg/mL was used for all test compounds and positive controls to observe the differences in the effects of different compounds at the same concentration. Results showed that the decline in ATP levels after treatment with compound **13** and **14** was noticeable compared to the positive controls. These results also provide good correlation with the conventional microdilution test (**Figure 2**).[26]

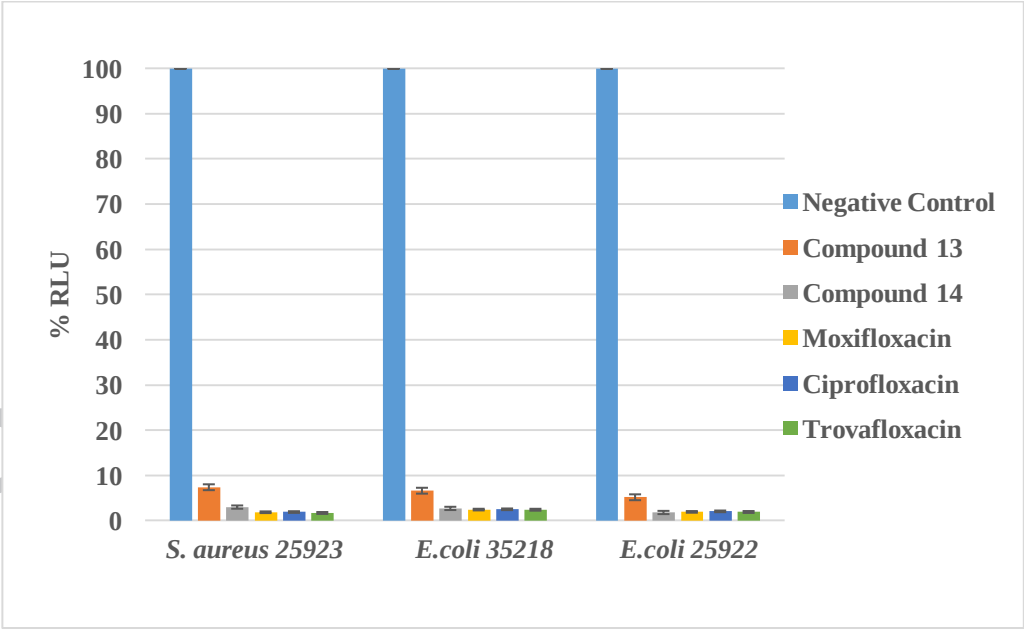


Figure 2. Relative luminescence activity of *S. aureus* (ATCC 25923), *E. coli* (ATCC 35218), and *E. coli* (ATCC 25922) in response to compounds **13** and **14** and moxifloxacin, ciprofloxacin, and trovafloxacin.

According to the ATP luminescence assay, compounds **13** and **14** are more effective against *E. coli* strains than *S. aureus*. Hence, we chose *E. coli* DNA gyrase as a target to show

a probable mechanism of action of the compounds.[27] Reactions were performed based on the supercoiling action of DNA gyrase on the relaxed DNA substrate provided in the kit (SKU TG2000G-3, TopoGen, USA) as pHOT1. Under proper conditions, gyrase (1U) supercoiled the relaxed DNA (pHOT1 - 0.25 µg) in 30 min at 37 °C. According to the gel electrophoresis, compounds **13** and **14** in similar concentration are effective against *E. coli* DNA gyrase. **Figure 3** shows the gel electrophoresis with (a, b) the two controls (c) relaxed DNA with DNA gyrase and (d) relaxed DNA with 1% DMSO**.** Compared to moxifloxacin and trovafloxacin, it is clear that compounds **13** and **14** are dose-dependent inhibitors (red arrows), but their effect on *E. coli* DNA gyrase is significantly higher than any other compound. Therefore, these two compounds were evaluated as DNA gyrase inhibitors. Gel documentation results show that compound **13** is less active than compound **14** and also that their activity is less than positive controls at 1.95 µg/mL. As a result, compounds **13** and **14** showed similar mechanism of action to the quinolone positive controls.

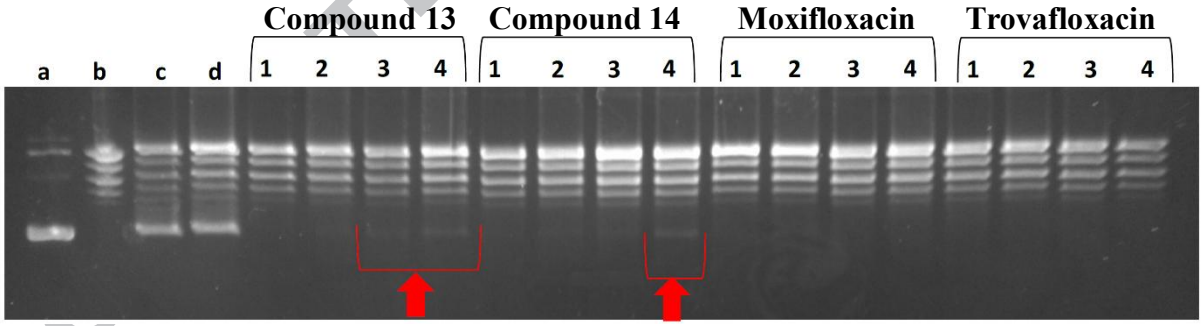


Figure 3: Inhibitory effect of compounds **13**, **14,** moxifloxacin, and trovafloxacin on the DNA gyrase. **a**: supercoiled DNA control, **b:** relaxed DNA (pHOT1) control, **c:** relaxed DNA with DNA gyrase, **d:** 1% DMSO, **1**. 15.6 µg/L, **2.** 7.8 µg /L, **3.** 3.9 µg/L, **4.** 1.95 µg/L

There are several conditions to be fulfilled for the successful development of a new drug. The drug candidate should indicate intrinsic pharmacological activity, be able to reach its target area, and not show toxicity. In this study, the toxicity of compounds **13** and **14**, was investigated via MTT assay, which is based on the reduction of yellow MTT dye by

metabolically active eukaryotic and prokaryotic cells to form the purple formazan product. This assay is mainly preferred in order to view cell viability and observe the growth of the cell culture.[28,29] The MTT assay was carried out using healthy NIH/3T3 mouse embryonic fibroblast cell lines (ATCC CRL165), which is recommended for cytotoxicity screening by ISO (10993-5, 2009).[30] Ciprofloxacin, trovafloxacin, and moxifloxacin were also subjected to MTT assay so as to compare their cytotoxicities with those of compounds **13** and **14**. The cytotoxicity results are presented in **Table 2**, in which it is shown that the IC_{50} values of compound **13** (121.81 µg/mL) and **14** (440.00 µg/mL) are approximately 8- and 54-fold higher than their highest MIC_{90} values (15.6 µg/mL for the compound **13** and 7.8 µg/mL for the compound **14**) determined against *K. pneumoniae* ATCC 700603, *L. monocytogenes* ATCC 7644, and *P. aeruginosa* ATCC 27853 (Table 1). Furthermore, compound **14** showed lower cytotoxicity than ciprofloxacin (IC_{50}=379.82 µg/mL), trovafloxacin (IC_{50}=244.28 µg/mL), and moxifloxacin (IC_{50}=305.77 µg/mL). These findings show that the antibacterial activity of the compound **14** is not due to general toxicity, but can be ascribed to selective action against bacteria (**Table 2**).

Table 2. IC_{50} of compounds **13, 14,** moxifloxacin, ciprofloxacin, and trovafloxacin on healthy mouse fibroblast cell lines NIH3T3 (ATCC CRL165).

Compound	13	14	Moxifloxacin	Ciprofloxacin	Trovafloxacin
IC_{50} (µg/mL)	121.81	440.00	379.82	305.77	244.28

Identification of mutagenic properties of new compounds is essential for safety, and thus, new drug candidates should be examined using models of genotoxicity, such as the Ames test.[31] The importance of this approach is quite high for new quinolone type DNA gyrase inhibitors because there are many studies reporting the mutagenic potency of the

compounds in this group.[15-20] These data have impacted medicinal chemistry efforts, ultimately leading to the production of inherently safer molecules.[32] Hence, in the current study, an Ames assay was performed to investigate the mutagenicity of compound **14,** which showed lower cytotoxicity than compound **13,** moxifloxacin, trovafloxacin, and ciprofloxacin did. In the Ames microplate format (MPF) assay, more than 25 positive wells were observed with our positive controls, and negative control wells also showed less than 8 positive wells in the presence and absence of S9 with TA98 and TA100, which complied with the requirements for the validation of the Ames MPF assay and also as described in previous studies.[33] Our results are presented in **Table 3.**

Table 3. AMES MPF results of the compounds

Compound	Concentration (mg/mL)	REVERTANTS Fold increase (over baseline)			
		TA 98		TA 100	
		S9+	S9-	S9+	S9-
14	0.156	0.00*	0.00*	0.00*	0.00***
	0.3125	0.11*	0.00*	0.00*	0.00***
	0.625	0.00*	0.00*	0.00*	0.00***
	1.25	0.00*	0.00*	0.00*	0.00***
	2.5	0.00*	0.00*	0.00*	0.00***
	5	0.00*	0.00*	0.00*	0.00***

* t test *p* value (unpaired 1-sided) < 0.05, *** t test *p* value (unpaired 1-sided) < 0.001

Compound **14** showed a baseline of 5.86 and 3.00 against TA98 with and without S9, respectively. Mentioned-fold increases over the baseline according to the criteria were not determined for compound **14** and significant results did not reach these values and did not show any dose–response tendencies. Compound **14** was also found to be non-mutagenic

against TA100 in the presence or absence of metabolic activation. It had a baseline of 7.42 with TA100 in the absence of S9 and 6.53 in the presence of S9. Fold inductions over baseline were less than 1.5 in each concentration of the compounds and there were no significant differences (**Figure 4**). As a result, compound **14** was accepted as non-mutagenic against TA98 and TA100 with and without metabolic activation. This finding improves the biological significance of the compound **14** as a DNA gyrase inhibitor.

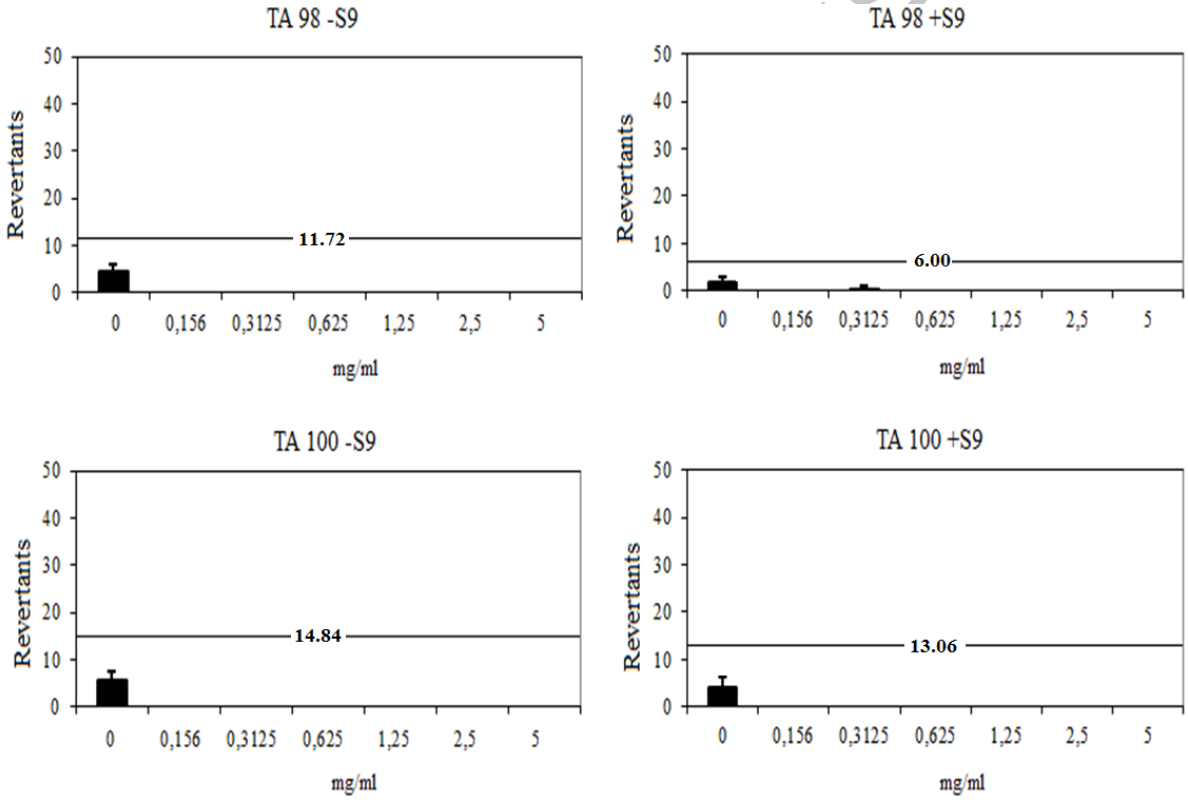


Figure 4. Dose–response curve of compound **14** against TA98 and TA100 in the presence and absence of S9 according to the AMES MPF test.

DNA gyrase binding pocket for fluoroquinolones is available from recently experimental structural data (protein crystal structure complexes) originating from different microorganisms (*S. pneumoniae* topo IV-DNALFX (levofloxacin) [pdb code: 3K9F], *A. baumanii* topo IV-DNAMOX (moxifloxacin) [pdb code: 2XKK] and *S. aureus* DNA gyrase-

DNA-CIP (ciprofloxacin) [pdb code: 2XCT]).[34-37] Among these X-ray crystal structures, *S. aureus* DNA gyrase-DNA-CIP (ciprofloxacin) [pdb code: 2XCT]) was retrieved from Protein Data Bank (PDB ID: 2XKK) (www.rcsb.org) and used in our docking studies. The docking of all synthesized derivatives and reference drugs showed correlate docking scores (**Table 4**), when compared with their MIC_{90} values. The docking score of compound **14** (-11.315 kcal.mol^{-1}) was very close to those of moxifloxacin (-11.747 kcal.mol^{-1}), ciprofloxacin (-11.705 kcal.mol^{-1}) and trovafloxacin (-11.387 kcal.mol^{-1}).

Table 4. Docking and glide scores of synthesized compounds (**3-14**), and reference drugs.

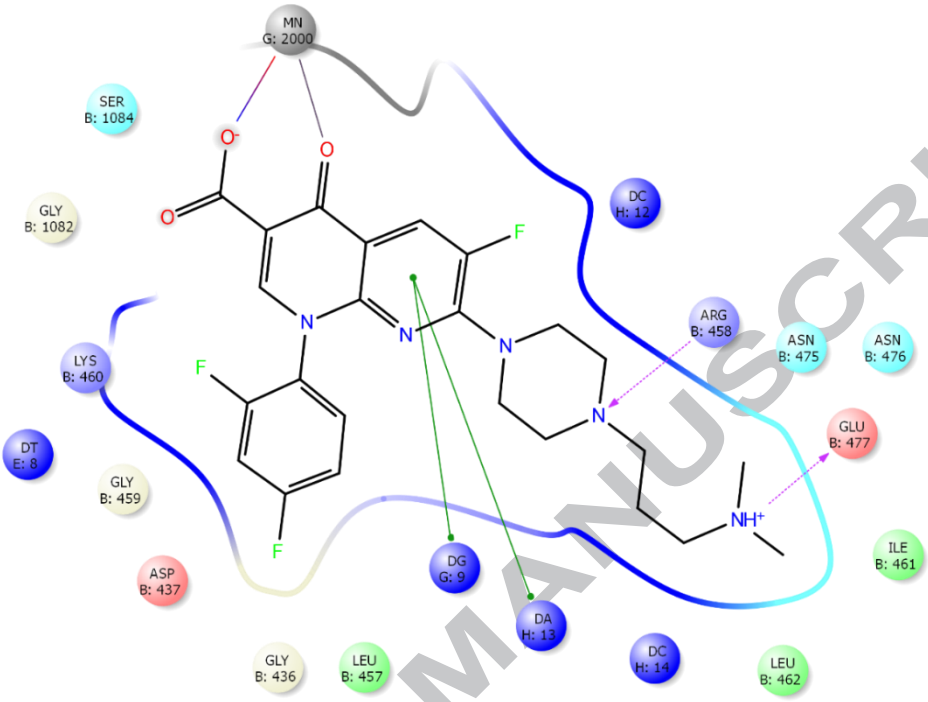
Compounds	*Docking Score*	*Glide Score*	*Glide Emodel*
3	-7.280	-7.280	-96.456
4	-7.255	-7.255	-91.223
5	-6.934	-6.934	-87.541
6	-6.561	-7.033	-93.460
7	-7.304	-7.462	-96.075
8	-8.060	-8.143	-104.209
9	-10.024	-10.029	-110.721
10	-9.964	-9.969	-110.169
11	-9.025	-9.030	-105.855
12	-9.125	-9.480	-102.205
13	-10.099	-10.099	-114.896
14	-11.315	-11.398	-132.413
Moxifloxacin	-11.747	-11.785	-109.336
Ciprofloxacin	-11.705	-11.705	-116.479
Trovafloxacin	-11.387	-11.387	-119.742

The docking poses including of **14** in the active site of the enzyme is presented in **Figures 5A** and **5B**. The figures reveal the disposition of protein side-chains, Mn^{+2} ion and DNA that form the drug binding pockets. Bax et al.[37] has demonstrated the importance of the metal ion (Mg^{+2} or Mn^{+2}) present in the binding pocket for establishing fluoroquinolone-metal-bridged coordinative interaction not only with the surrounding amino acid residues of the protein but

also with the neighboring DNA base pairs. It has also been stated that the interaction between the Mn^{+2} ion and the oxygen at 3rd position of fluoroquinolone greatly accelerates rates of enzyme-mediated DNA cleavage.[37,38] We observed that Mn^{+2} ion interacts with oxygens at both 3rd and 4th positions of compound **14** as seen **Figures 5A** and **5B**. This interaction has also been reported for ciprofloxacin in previous studies.[35,37,39] Additionally, it has been shown that binding to DNA cleavage is essential for fluoroquinolones.[35,37,39] Our docking study reveals that compound **14** binds to DNA cleavage as similar as ciprofloxacin does.[37] The naphthyridine core ring establishes π-π interactions with guanine (DG G:9) and adenine (DA H:13) DNA bases (**Figures 5A** and **5B)**. The 7th position of core ring has been displayed as a key attachment point in fluoroquinolones, and piperazinyl and pyrrolidinyl groups have been reported as the most frequent fragments attached at this position.[35,39] In our study, we observed that nitrogen at the 4th position of piperazine forms a hydrogen bond with amino group of Arg458 by acting as a hydrogen acceptor. In addition, nitrogen of propylamino side chain is in an interaction with Glu477 by forming a hydrogen bond. As a result of docking study, it can be declared that compound **14** is very compatible with the active site of *S. aureus* DNA gyrase (pdb code: 2XCT).

In summary, preliminary evaluation of new 1,4-dihydro[1,8]naphthyridine derivatives as antibacterial agents resulted in promising findings. Compound **14** showed a good antibacterial profile and DNA gyrase inhibition. Furthermore, this compound did not show cytotoxicity and mutagenicity, which is a well-known side effect related to this class of antibiotics. A docking study indicated significant interactions between compound **14** and *S. aureus* DNA gyrase. Consequently, findings of the present study will not only direct our research group to further studies, but also may have an impact on medicinal chemists, encouraging them to synthesize more effective and safer compounds bearing chemical structures similar to that of compound **14**.

(A)



(B)

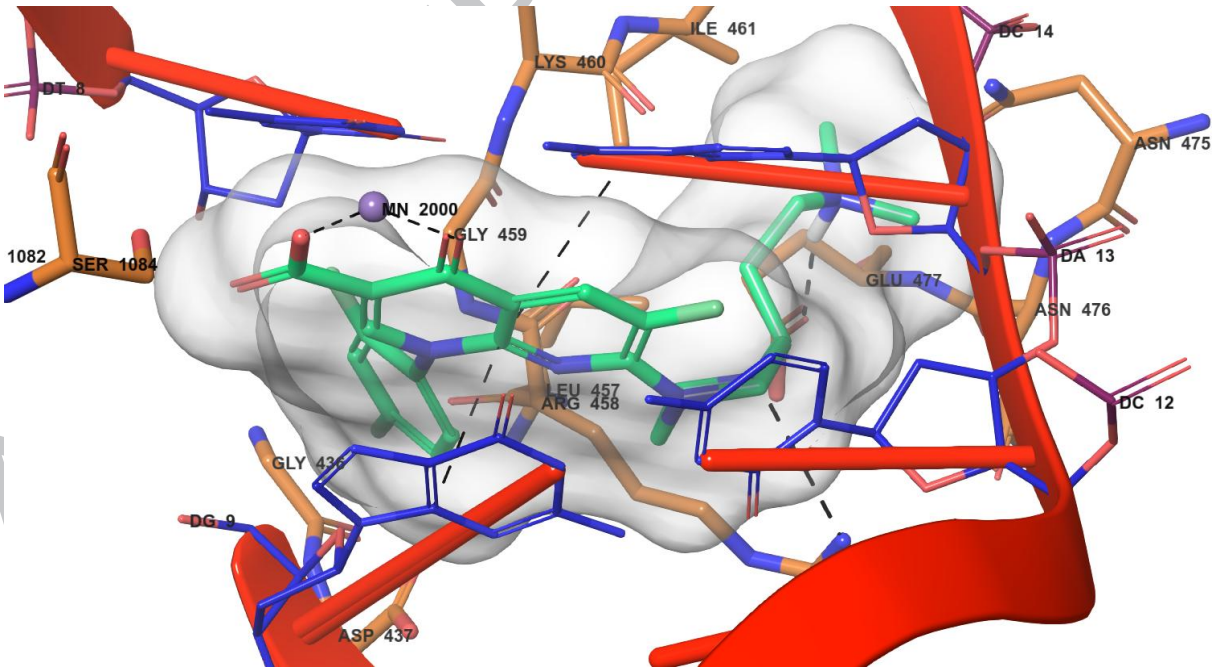


Figure 5. (A) Two-dimensional model of interactions of compound **14** in DNA gyrase (PDB code 2XCT) active site. **(B)** The interacting mode of compound **14** in the active region. The inhibitor colored green and the important amino acid residues colored orange in the active site of the enzyme are presented by tube model. The important DNA bases are colored blue with thin tube model. Mn^{+2} ion is displayed with purple color.

Acknowledgements

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22. Initially, ethyl 7-chloro-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (**1**) were hydrolysed to 7-chloro-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**2**) in 10% HCl. Then, compounds **1** and **2** in dry acetone were reacted with corresponding benzazol-2-thiol derivative or N,N-dimethyl-(3-piperazin-1-yl)propan-1-amine to give the 12 new 7-substituted-1-(2,4-difluorophenyl)-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid or carboxylic acid ethyl ester (**3-14**).

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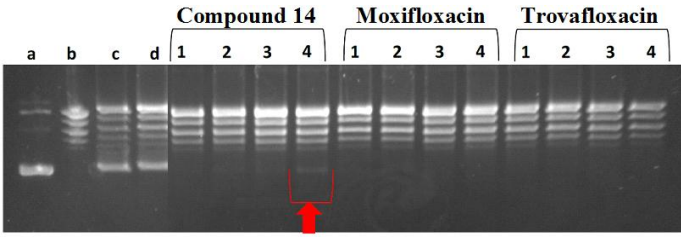
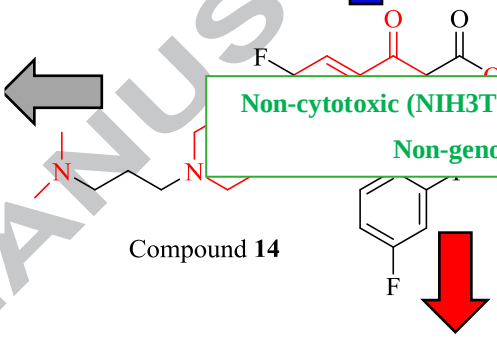
NEW 1,4-DIHYDRO[1,8]NAPHTHYRIDINE DERIVATIVES AS DNA GYRASE INHIBITORS

A significant MIC value (1.95 µg/mL) against *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC 35218 and *Escherichia coli* ATCC 25922



Escherichia coli DNA gyrase inhibition

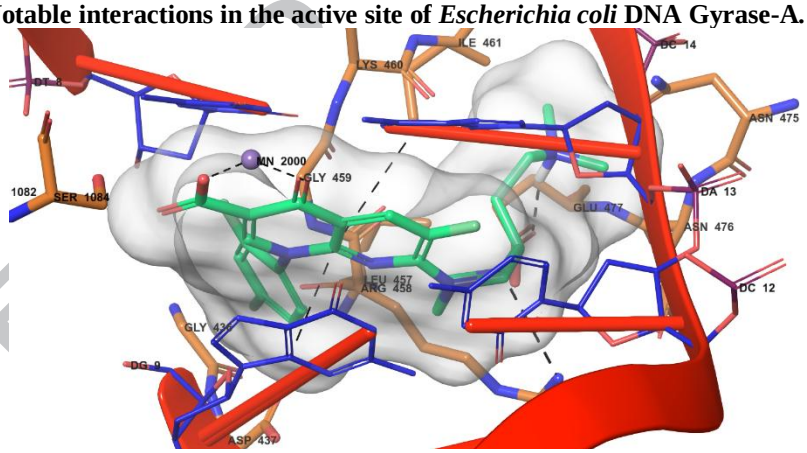


Compound **14**

Non-cytotoxic (NIH3T3 IC_{50}: 440 µg/mL)

Non-genotoxic

Notable interactions in the active site of *Escherichia coli* DNA Gyrase-A.



Highlights

- ✓ New 1,4-Dihydro[1,8]naphthyridine derivatives were synthesized as potential antibacterial and DNA Gyrase inhibitory agents.
- ✓ Compounds **13** and **14** displayed a good antibacterial profile in both Broth Microdilution and ATP Luminescence assays.
- ✓ These compounds caused an inhibition on *E. coli* DNA gyrase enzyme.
- ✓ Both of the compounds did not display any cytotoxicity and genotoxicity.
- ✓ Interactions between compound **14** and *S. aureus* DNA gyrase were determined by docking studies.